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# Relaxant Effect of *Thymus vulgaris* on Guinea-pig Tracheal Chains and its Possible Mechanism(s)

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***Thymus vulgaris*** **for the treatment of respiratory diseases is indicated widely, and relaxant effects on smooth muscle have been shown previously. In the present study, the relaxant effects of macerated and aqueous extracts of** ***Thymus vulgaris*** **on tracheal chains of guinea-pigs were examined using cumulative concentrations of macerated and aqueous extracts in comparison with saline (as the negative control) and theophylline (as the positive control). The relaxant effects of four cumulative concentrations of macerated and aqueous extracts (0.25, 0.5, 0.75 and 1.0 g %) in comparison with saline (as the negative control) and four cumulative concentrations of theophylline (0.25, 0.5, 0.75 and 1.0 mM; as the positive control) were examined for their relaxant effects on precontracted tracheal chains of guinea-pig by 60 mM KCl and 10 μM methacholine in two different conditions: non-incubated tissues and incubated tissues with 1 μM propranolol and 1 μM chlorphenamine. There were significant correlations between the relaxant effects and the concentrations for both extracts and theophylline in all experimental groups ($p < 0.01$ to $p < 0.001$). These results demonstrated a potent relaxant effect of** ***Thymus vulgaris*** **on guinea-pig tracheal chains that was comparable to theophylline at the concentrations used. Copyright © 2006 John Wiley & Sons, Ltd.**

*Keywords: Thymus vulgaris*; bronchodilatory; guinea-pig trachea.

## INTRODUCTION

*Thymus vulgaris* L. is a grassy annual plant that grows in many areas of the world. It has an agreeable aromatic smell and a warm pungent taste; the fragrance is due to an essential oil, which gives it its flavouring value for culinary purposes, and is also the source of its medicinal properties (Mossa *et al.*, 1987).

The main active constituents include: terpenes, phenols, thymol, carvacrol, glycosides of phenolic monoterpenoids, eugenol and aliphatic alcohols, the flavonoids thymonin, cirsilineol, and 8-methoxycirsilineol, biphenyl compounds of monoterpenoid origin, caffeic and rosmarinic acids and saponins (ESCOP, 1997). Other constituents include tannins, labiatic acid, ursolic acid and oleanolic acid. Thyme also contains apigenin, luteolin and 6-hydroxyluteolin glycosides, as well as di-, tri- and tetramethoxylated flavones (Mossa *et al.*, 1987).

Thyme extract has been used orally to treat dyspepsia and other gastrointestinal disturbances; coughs due to colds, bronchitis and pertussis; and laryngitis and tonsillitis (as a gargle). Topical applications of thyme extract have been used in the treatment of minor wounds, the common cold, disorders of the oral cavity and as an antibacterial agent in oral hygiene. Both the essential oil and thymol are ingredients of a number of proprietary drugs including antiseptic and healing ointments, syrups for the treatment of respiratory disorders, and preparations for inhalation (Mossa *et al.*, 1987). It has also been used to improve digestion (Stecher, 1968) and to treat pertussis, stomatitis and halitosis (ESCOP, 1997).

Previous studies have shown different therapeutic effects of this plant including the symptoms of bronchitis, whooping cough and catarrh of the upper respiratory tract. The relaxant effect of this plant on tracheal and ileal smooth muscle has been shown (Meister *et al.*, 1999), and the relaxant effect of related plants on tracheal and ileal smooth muscle of guinea-pig has been also reported previously (Reiter and Brandt, 1985).

*In vitro* studies have shown that both thyme essential oil and thymol have antifungal activity against a number of fungi, including *Cryptococcus neoformans*, *Aspergillus*, *Saprolegnia* and *Zygorhynchus* species (Llewellyn *et al.*, 1981; Tantaouielaraki and Errifi, 1994; Vollon and Chaumont, 1994; Macchioni *et al.*, 1999). Both the essential oil and thymol had antibacterial activity against *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli* and a number of other bacterial species (Janssen *et al.*, 1987; Juven *et al.*, 1994). As an antibiotic, thymol is 25 times as effective as phenol, but less toxic (Czygan, 1989).

Therefore, in the present study, the relaxant effect of macerated and aqueous extracts from *Thymus vulgaris* on guinea-pig tracheal chains and its possible mechanism(s) was examined.

## MATERIALS AND METHODS

**Plant and extracts.** *Thymus vulgaris* was collected from mountainous areas of central eastern Iran. A voucher specimen was preserved in the Herbarium of the School

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of Agriculture, Ferdowsi University (Herbarium no: 153-2613-2). The aqueous extract was prepared as follows: Fifty grams of the chopped, dried plant was extracted with 300 mL distilled water by Soxhlet apparatus. For the macerated extract, the same amount of plant was macerated with 300 mL distilled water (on a shaker) for 48 h. The solvent of both extracts was then removed under reduced pressure and distilled water was added so that the plant ingredient concentration in the final aqueous extract was 10% g % in all extracts.

**Tissue preparations.** Male Dunkin-Hartley guinea-pigs (400–700 g) were killed by a blow on the neck and the tracheas were removed. Each trachea was cut into 10 rings (each containing 2–3 cartilaginous rings). All the rings were then cut open opposite the tracheal muscle, and sutured together to form a tracheal chain (Holroyde, 1986). Tissue was then suspended in a 10 mL organ bath (organ bath 61300, Bioscience Palmer-Washington, Sheerness, Kent UK) containing Krebs-Henseleit solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, $KH_2PO_4$ 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11.

The Krebs solution was maintained at 37 °C and gassed with 95% $O_2$ and 5% $CO_2$. The tissue was suspended under an isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

**Protocols.** The relaxant effects of four cumulative concentrations of macerated and aqueous extracts (0.25, 0.5, 0.75 and 1.0 g %), four cumulative concentrations of theophylline anhydrous (Sigma Chemical Ltd UK) (0.25, 0.5, 0.75 and 1.0 mM) as a positive control, and saline as a negative control (1 mL) were examined. To produce different concentrations of macerated and aqueous extracts, 0.25 mL of 10 g % concentrated extracts were added to a 10 mL organ bath, respectively, four times. For theophylline, 0.25 mL of 10 mM concentrated solution was added to the organ bath four times. The consecutive volumes were added to the organ bath at 5 min intervals.

In each experiment the effect of four cumulative volumes from each extract, four cumulative volumes from theophylline, or saline on the contracted tracheal smooth muscle was measured after exposing the tissue to the solution for 5 min. A decrease in tone was considered as a relaxant (bronchodilatory) effect and expressed as a positive percentage change in proportion to the maximum contraction and an increase in tone was considered as a contractile (bronchoconstrictory) effect, which was expressed as a negative percentage change (Martin *et al.*, 1994).

The relaxant effect of different solutions was tested with two different experimental designs as follows:

1. On tracheal chains contracted by 60 mM KCl (group 1 experiments, $n = 5$).
2. On non-incubated tracheal chains contracted by 10 μM methacholine hydrochloride (Sigma Chemical Ltd UK), (group 2 experiments, $n = 7$).
3. On incubated tracheal chains with 1 μM propranolol hydrochloride (Sigma Chemical Ltd UK) and 1 μM chlorphenamine maleate (Sigma Chemical Ltd UK) 30 min prior to beginning and during the testing relaxation of different solutions. In this series of experiments, tracheal chains were also contracted by 10 μM methacholine hydrochloride (group 3 experiments, $n = 5$).

The relaxant effect of theophylline was examined only on groups 1 and 2. The relaxant effects in the three groups of experiments were examined in three different series of tracheal chains. All the experiments were performed randomly with a 1 h resting period of tracheal chains between each two experiments while washing the tissues every 15 min with Krebs solution. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and were measured after fixation.

**Statistical analysis.** All data were expressed as mean ± SEM. Data of relaxant effects of different concentrations of extracts were compared with the results of negative and positive control using ANOVA. The data of relaxant effect obtained in three groups of experiments were also compared using ANOVA. The relaxant effect of two extracts and theophylline were related to the concentrations using least squares regression. Significance was accepted at $p < 0.05$.

## RESULTS

### Relaxant (bronchodilatory) effect

In group 1 experiments only the three highest concentrations of theophylline and two higher concentrations of aqueous extract showed significant relaxant effects compared with those of saline ($p < 0.05$ to $p < 0.001$). The effects of the two highest concentrations of the macerated extract and the three highest concentrations of aqueous extract were significantly lower than those of theophylline ($p < 0.01$ to $p < 0.001$). In addition, the effects of the last concentration of the aqueous extract was significantly higher than that of the macerated extract in this group ($p < 0.05$) (Table 1).

In group 2 both extracts from *Thymus vulgaris* and theophylline showed relatively potent and concentration-dependent relaxant effects on tracheal chains of guinea-pig. The relaxant effects of the most concentrations of extracts and theophylline were significantly higher than those of saline ($p < 0.05$ to $p < 0.001$). Only the lowest concentration of both extracts and theophylline did not show significant relaxant effects (Table 2). However, the effects of the highest concentrations of both extracts in group 2 were significantly lower than that of theophylline ($p < 0.001$ for both cases) (Table 2). There were no significant differences in the effect of the different concentrations between two extracts in group 2.

In group 3, the extracts of *Thymus vulgaris* did not show any significant relaxant effect compared with the effect of saline (Table 3).

### Comparison of the relaxant effect between three groups of experiments

The relaxant effects of most concentrations of both extracts in group 2 were statistically greater than those

**Table 1. Relaxant effect of two different extracts from *Thymus vulgaris* in comparison with negative control (saline) and positive control (theophylline) in group 1 experiments (contracted tracheal chains with 60 mM KCl, $n = 5$)**

| Different concentration | Saline | Macerated extract | Aqueous extract | Theophylline |
|---|---|---|---|---|
| 0.25 | – | 2.00 ± 1.490 NS ns | 0.00 ± 0.00 NS ns nS | –3.58 ± 1.57 NS |
| 0.5 | – | 8.00 ± 5.06 NS ns | 0.00 ± 0.00 NS + nS | 17.98 ± 5.35* |
| 0.75 | – | 10.00 ± 6.33 NS ++ | 19.4 ± 5.72* + nS | 46.80 ± 6.54*** |
| 1 | 1.34 ± 0.65 | 19.8 ± 9.71 Ns +++ | 56.8 ± 8.09*** + * | 80.60 ± 4.50*** |

Values are presented as mean ± SEM. Statistical differences between the effect of extracts and negative control (saline); NS: non-significant difference between the effect of extracts and negative control (saline). *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$. Statistical differences between the effect of extracts and positive control (theophylline); ns: non-significant difference between the effect of extracts and positive control (theophylline). +; $p < 0.05$; ++; $p < 0.01$, +++; $p < 0.001$. Statistical differences between the effect of two extracts; nS: non-significant difference between the effect of two extracts. *; $p < 0.05$. The unit of concentration for extracts was g % and for theophylline was mM.

**Table 2. Relaxant effect of two different extracts from *Thymus vulgaris* in comparison with negative control (saline) and positive control (theophylline) in group 2 experiments (non incubated preparation contracted by 10 μM methacholine, $n = 7$)**

| Different concentration | Saline | Macerated extract | Aqueous extract | Theophylline |
|---|---|---|---|---|
| 0.25 | – | 5.43 ± 2.94 NS ns | 4.29 ± 2.97 NS ns nS | –1.89 ± 0.31 NS |
| 0.5 | – | 13.86 ± 3.08* ns | 11.93 ± 3.39* ns nS | 12.41 ± 1.88* |
| 0.75 | – | 24.14 ± 4.69*** ns | 22.71 ± 3.05** ns nS | 33.32 ± 3.49*** |
| 1 | 0.98 ± 0.54 | 38.86 ± 4.37*** +++ | 32.43 ± 7.11*** +++ nS | 74.61 ± 5.15*** |

For abbreviations see Table 1.

**Table 3. Relaxant effect of two different extracts from *Thymus vulgaris* in comparison with negative control (saline) and positive control (theophylline) in group 3 experiments (incubated preparations with 1 μM propranolol and 1 μM cholorpheniramine contracted by 10 μM methacholine, $n = 5$)**

| Different concentration | Saline | Macerated extract | Aqueous extract |
|---|---|---|---|
| 0.25 | – | 0.00 ± 0.00 NS | 2.40 ± 1.50 NS nS |
| 0.5 | – | 0.20 ± 0.20 NS | 4.40 ± 2.80 NS nS |
| 0.75 | – | 4.60 ± 2.23 NS | 7.00 ± 4.64 NS nS |
| 1 | 1.12 ± 0.58 | 5.4 ± 2.36 NS | 9.4 ± 5.5 NS nS |

For abbreviations see Table 1.

of group 3 experiments ($p < 0.05$ to $p < 0.001$). The relaxant effects of most concentrations of the aqueous and macerated extracts in group 2 were not significantly higher than those of group 1. However, the effect of the highest concentration of the aqueous extract in the group 1 experiments was significantly greater than those of groups 2 and 3. In addition, there were no significant differences in the effect of all concentrations of theophylline between groups 1 and 2 (Fig. 1).

### Correlation between concentrations of solutions and their relaxant effect

There were significant positive correlations between the relaxant effects of both extracts and theophylline with concentrations of the solutions in all three experimental groups ($p < 0.01$ to $p < 0.001$) (Table 4).

**Table 4. Correlations ($r$) between the relaxant effects of two different extracts from *Thymus vulgaris* and theophylline with concentration in three groups of experiments**

| Different substances | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| Macerated extract | 0.714*** | 0.791*** | 0.565** |
| Aqueous extract | 0.842*** | 0.696*** | 0.613*** |
| Theophylline | 0.951*** | 0.936*** | – |

Statistical significances; NS: non-significant difference, *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$.

## DISCUSSION

In this study the relaxant (bronchodilatory) effects of the macerated and aqueous extracts from *Thymus*

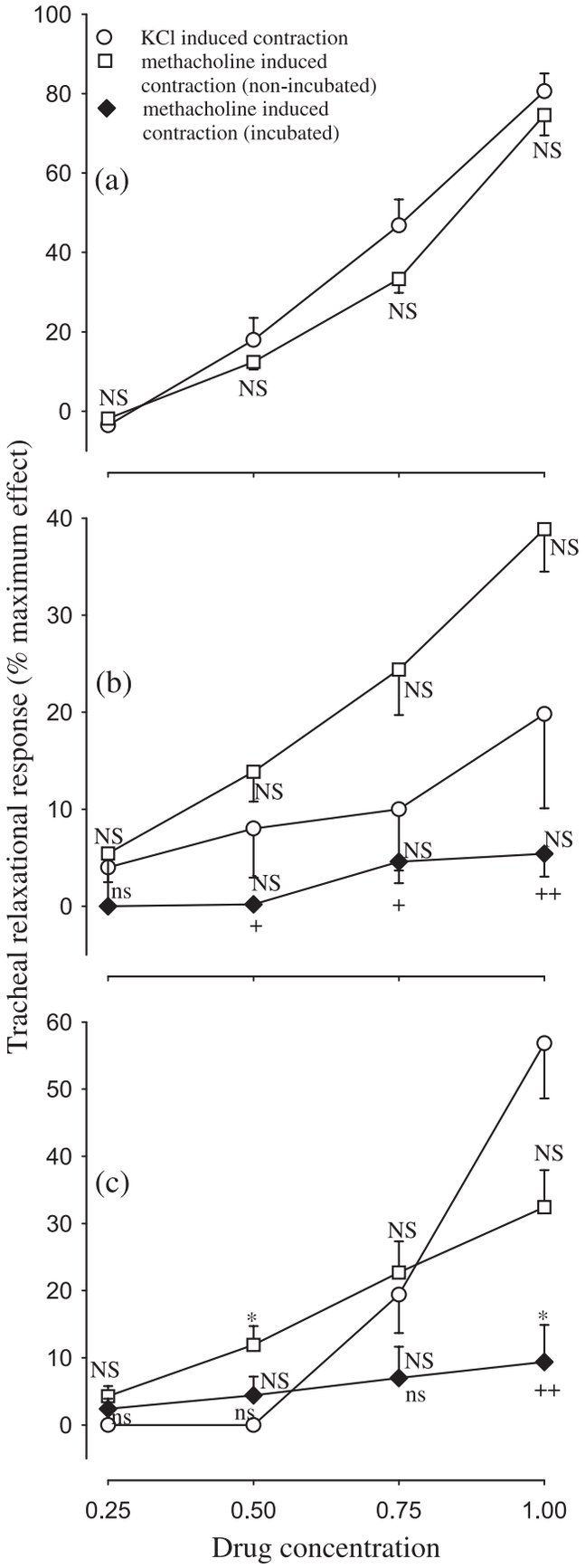


**Figure 1.** Concentration response curves of the relaxant effect of theophylline, macerated extract (b), and aqueous extract (c) from *Thymus vulgaris* in three groups of experiments (group 1; KCl induced contraction on non-incubated tracheal chains (○, $n = 5$), group 2; methacholine induced contraction on non-incubated tracheal chains (□, $n = 7$), and group 3 experiments methacholine induced contraction on incubated tracheal chains of guinea pig with propranolol and chlorphenamine (◆, $n = 5$) Statistical differences in the relaxant effect of different substances between group 1 with those of groups 2 and 3; NS: non-significant difference, *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.002$. Statistical differences in the relaxant effect of different substances between groups 2 and 3; ns: non-significant difference, +; $p < 0.05$, ++; $p < 0.01$.

*vulgaris* in comparison with saline as a negative control and theophylline as a positive control were studied. In group 1 experiment (contracted tracheal chains by KCl) only the two highest concentrations of theophylline and the highest concentration of aqueous extract showed relaxant effect on tracheal smooth muscle. The macerated extract and saline did not show any relaxant effect in this group of experiments. However, both extracts from *Thymus vulgaris* showed relatively potent relaxant effects compared with the effect of saline in group 2 experiments. In group 3, the extracts of *Thymus vulgaris* did not show any significant relaxant effect compared with that of saline. The effect of theophylline was not examined in group 3 experiments.

The relaxant effect of both extracts and theophylline was concentration dependent. There were positive correlations between increasing concentrations and the relaxant effects of both extracts in all three groups of experiments. The relaxant effects of all concentrations of macerated extracts were very similar to those of aqueous extract in groups 2 and 3. In addition, the effects of both extracts in group 2 experiments were comparable to that of theophylline.

The relaxant effect of different extracts from *Thymus vulgaris* on tracheal chains of guinea pigs might be due to several different mechanisms including stimulation of $\beta$-adrenergic receptors, inhibition of histamine $H_1$ receptors or an anticholinergic property of this plant, because the relaxant effect of $\beta_2$-stimulatory (Martin *et al.*, 1994; Linden *et al.*, 1993), histamine $H_1$ receptor inhibitory (Popa *et al.*, 1984) and anticholinergic drugs (Loenders *et al.*, 1992) have been shown in previous studies. To evaluate the contribution of $\beta$-adrenergic stimulatory and/or the $H_1$ histamine blocking effect of macerated and aqueous extracts from this plant on their bronchodilatory effects, the effects of these extracts on tracheal chains with inhibited $\beta$-adrenergic and histamine $H_1$ receptors by propranolol and chlorphenamine respectively, were re-examined in group 3 experiments. The relaxant extracts of the plant did not show any relaxant effect in group 3 experiments. The relaxant effects of most concentrations of both extracts from *Thymus vulgaris* obtained in the group 3 experiments were significantly lower than those of group 2. These findings suggest probable $\beta$-adrenergic stimulatory and/or histamine $H_1$ blocking properties of the plant extracts that may contribute to their relaxant effect on the tracheal chains of guinea-pig. The enhanced relaxant effect of both extracts in higher concentrations and positive correlations between their effects and concentrations support the competitive antagonist effect of the extracts on $\beta$-adrenoceptors and/or histamine $H_1$ receptors.

The absence of obvious relaxant effects of a macerated extract from *Thymus vulgaris* in group 1 and the relatively potent relaxant effect of this extract in group 2 experiments may indicate an opening effect of these fractions on potassium channels because the bronchodilatory effect of potassium channel opening

has been demonstrated previously (Buckle *et al.*, 1993). If the macerated extract had a potassium channel opening effect, they would not have a relaxant effect on tracheal chains contracted by KCl, while they could show a relaxant effect when the tracheal chain was contracted by metacholine. In fact, the results of group 2 may support this effect of the macerated extract. While KCl affects calcium channels (Perez-Guerrero *et al.*, 1997) and with regard to the bronchodilatory effect of calcium channel blockers (Miyahara *et al.*, 1993; McCaig and DeJonckeere, 1993), another explanation for these findings is the absence of a blocking effect of this extract on calcium channels.

Although the macerated extract did not show a significant relaxant effect but the increasing effect in higher concentration suggested an inhibitory effect on calcium channels of guinea-pig tracheal chains. The positive correlation between its effect and the concentration support this suggestion. However, the significant relaxant effect of aqueous extract in the group 1 experiment may suggest the absence of any effect on potassium channels and/or calcium channels blocking effect for this extract.

The results of this study confirmed those of Reiter and Brandt (1985) and Meister *et al.* (1999) indicating the relaxant effect of this plant on tracheal and ileal smooth muscles. However, in the present study some mechanisms responsible for the relaxant effect of the plant including $\beta$ adrenoceptor stimulatory, histamine $H_1$ inhibitory, calcium channel inhibitory and potassium channel opening effects were also explored.

The spasmolytic and antitussive activity of thyme has been most often attributed to its constituents thymol and carvacrol, which make up a large percentage of the volatile oil (Reiter and Brandt, 1985). Although these compounds have been shown to prevent contractions induced in the ileum and the trachea of the guinea-pig, by histamine, acetylcholine and other reagents, the concentration of phenolics in aqueous preparations of the drug is insufficient to account for this activity (Van Den Broucke, 1980; Van Den Broucke and Lemli, 1981). Our previous study (Boskabady and Jandaghi, 2003) showed a potent effect for carvacol, which is one constituent of *Thymus vulgaris.* Therefore, the carvacol content of the plant may be responsible for its relaxant effect on tracheal chains. Experimental evidence also suggests that the *in vitro* spasmolytic activity of thyme preparations is due to the presence of polymethoxyflavones (Van Den Broucke and Lemli, 1983). *In vitro* studies have shown that flavones and thyme extracts inhibit the responses to agonists of specific receptors such as acetylcholine, histamine and L-norepinephrine, as well as agents whose actions do not require specific receptors, such as barium chloride (Van Den Broucke and Lemli, 1983). The flavones of thyme were found to act as noncompetitive and nonspecific antagonists (Van Den Broucke and Lemli, 1983); they were also shown to be $Ca_2$-antagonists and musculotropic agents that act directly on smooth muscle (Van Den Broucke and Lemli, 1983).

The relaxant effect of the plant is not due to its main constituent, thymol, because our previous study failed to show any relaxant effect for thymol on tracheal chains of guinea-pig (Boskabady *et al.*, 1998). However, more studies are required to reveal the different therapeutic effect, effective substance(s) and extract mechanism(s) of action of relaxant effect *Thymus vulgaris.*

Although both macerated and aqueous extracts are aqueous in nature, the result of the present study showed some differences in their effect. The differences in the effect of the two extracts could be due to variation in their constituents due to the method of extraction.

With regard to the existence of airway inflammation in the tracheobronchial tree of asthmatic patients, *Thymus vulgaris* might also have an antiinflammatory effect, which will contribute to the therapeutic effect of this plant on asthma. In fact, the antioxidant (Haraguchi *et al.*, 1996) effects of this plant have been shown.

A secretomotor activity has also been suggested for thyme oil which has been associated with a saponin extract from *Thymus vulgaris* (Vollmer, 1932). Stimulation of ciliary movements in the pharynx mucosa of frogs treated with diluted solutions of thyme oil, thymol or carvacrol have also been reported (Freytag and ber den Einflu, 1933). Furthermore, an increase in mucus secretion of the bronchi after treatment with thyme extracts has been observed (Schilf and Einfluss von, 1932). Muller-Limmroth and Frohlich (1980) have suggested a protective effects due to mucous layers in the hypopharynx and spasmolytic, secretolytic and bactericide effect (Muller-Limmroth and Frohlich, 1980). In contrast, Lemiere *et al.* (1996) have reported occupational asthma due to exposure of the subjects to thyme. It is also reported that plants belonging to the Labiatae family seem to show cross-sensitivity on the basis of clinical history and *in vitro* and *in vivo* test results (Benito *et al.*, 1996). However, the effect of *Thymus vulgaris* on airway inflammation existing in asthma and other respiratory disease needs further investigation.

In conclusion, the results of this study indicate a relatively potent relaxant effect of *Thymus vulgaris* on tracheal chains of guinea-pig, which was comparable to that of theophylline. The results suggested that the relaxant effect of this plant could be due to $\beta$-adrenoceptor stimulatory and/or histamine ($H_1$) receptor inhibitory effect, a possible potassium channel opening effect for the macerated extract and an inhibitory effect of the aqueous extract on calcium channels is also postulated.

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